DATA SUPPLEMENT

A Combined Molecular and Clinical Prognostic Index for Relapse and Survival in Cytogenetically Normal Acute Myeloid Leukemia

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Table of Contents

SUPPLEMENTARY FIGURES 9

SUPPLEMENTARY TABLES (pages 16-26)

SUPPLEMENTARY REFERENCES 27

SUPPLEMENTARY TEXT

PATIENTS AND METHODS

Patient treatment for the AMLCG99 study:

The trial was approved by the ethics committees of the participating institutions and was performed in accordance with the Declaration of Helsinki. All patients were randomly assigned to one course of induction therapy with either TAD (thioguanine 100 mg/m^2 q 12 hrs orally days 3-9; cytarabine [Ara-C] 100 mg/m^2/d continuous i.v. infusion days 1 and 2; Ara-C 100 mg/m^2 q 12 hrs 30 min. i.v. infusion days 3-8; daunorubicin 60 mg/m^2 60 min. i.v. infusion days 3-5) or HAM (Ara-C 3 g/m^2 q 12 hrs i.v. in patients <60 years or 1 g/m^2 q 12 hrs i.v. in patients ≥60 years infusion over 3 hrs days 1-3; mitoxantrone 10 mg/m^2/d 60 min. i.v. infusion days 3-5). A second induction therapy with HAM was given to all patients <60 years and to patients ≥60 years with ≥5% bone marrow blasts one week after the first induction course. All patients underwent consolidation therapy with TAD 2 to 4 weeks after achievement of a complete remission (CR). Patients <60 years were randomly assigned for postremission therapy with prolonged maintenance (monthly chemotherapy with cytarabine 100 mg/m^2 q 12 hrs s.c. days 1-5 combined with monthly alternating either daunorubicin 45 mg/m^2 60 min. i.v. infusion days 3 and 4, thioguanine 100 mg/m^2 q 12 hrs orally days 1-5 or cyclophosphamide 1 g/m^2 i.v. day 3) or autologous stem cell transplantation (SCT) after myeloablative therapy with busulfan 4x1 mg/kg/d p.o. and cyclophosphamide 60 mg/kg 1h i.v. When an HLA-compatible family donor was available and there were no medical contraindications, allogeneic transplantation was performed. Patients without a family donor were offered allogeneic transplantation only in case of relapse. All patients ≥60 years underwent consolidation therapy with TAD and a three year maintenance therapy with an alternating regimen of AD (cytarabine 100 mg/m^2 q 12 hrs s.c. days 1-5; 45 mg/m^2 daunorubicin 60 min i.v. infusion days 3 and 4) – AT (cytarabine 100 mg/m^2 q 12 hrs s.c. days 1-5; thioguanine 100 mg/m^2 q 12 hrs orally days 1-5) – AC (cytarabine 100 mg/m^2 q 12 hrs s.c. days 1-5; cyclophosphamide 1 g/m^2 i.v. day 3).

Previous results showed no outcome difference between the randomized induction regimen and the randomized postremission treatment of this cohort[1].

Patient treatment for the CALGB external validation cohort:

Patients with cytogenetically normal acute myeloid leukemia (CN-AML) <60 years were treated on Cancer and Leukemia Group B (CALGB) trials 9621 or 19808. Patients enrolled on CALGB 19808 (n=175) were randomly assigned to receive induction chemotherapy with cytarabine, daunorubicin, and etoposide with or without PSC-833 (valspodar), a multidrug resistance protein inhibitor[2]. On achievement of CR, patients were assigned to intensification with high-dose cytarabine and etoposide for stem-cell mobilization followed by myeloablative treatment with busulfan and etoposide supported by peripheral blood autologous SCT. Patients enrolled on CALGB 9621 (n=104) were treated similarly to those on CALGB 19808, as previously reported[3,4]. Older patients (≥60 years) were all treated with cytarabine/daunorubicin-based induction therapy followed by cytarabine-based consolidation therapy. Patients on CALGB 8525 (n=24) were treated with induction chemotherapy consisting of cytarabine in combination with daunorubicin and were randomly assigned to consolidation with different doses of cytarabine followed by maintenance treatment[5]. Patients on CALGB 8923 (n=25) were treated with induction chemotherapy consisting of cytarabine in combination with daunorubicin and were randomly assigned to receive postremission therapy with cytarabine alone or in combination with mitoxantrone[6]. Patients on CALGB 9420 (n=6) and 9720 (n=113) received induction chemotherapy consisting of cytarabine in combination with daunorubicin and etoposide, with (CALGB 9420) or with/without (CALGB 9720) the multidrug resistance protein modulator PSC-833[7,8]. Patients on CALGB 9420 received postremission therapy with cytarabine (2 g/m^2/d) alone, and patients on CALGB 9720 received a single cytarabine/daunorubicin consolidation course identical to the induction regimen and were then randomly assigned to low-dose recombinant interleukin-2 maintenance therapy or none[9]. Patients on CALGB 10201 (n=82) received induction chemotherapy consisting of cytarabine and daunorubicin, with or without the *BCL2* antisense oblimersen sodium. The consolidation regimen included two cycles of cytarabine (2 g/m^2/d) with or without oblimersen[10].

Statistical methods:

For Cox regression analyses continuous variables were not categorized because this would have reduced the statistical power[11]. White blood count (WBC), platelet count and lactate dehydrogenase (LDH) levels were evaluated on the log scale because of their skewed distributions. Variables with more than 10% missing values were excluded from multivariable Cox regression.

We checked the proportional hazard assumption in the full and final models for overall survival (OS) and relapse-free survival (RFS) based on scaled Schoenfeld residuals using the function cox.zph of the R-package "survival". All variables showed p-values larger than 0.01 for a potential interaction with time, except for *NPM1* mutation in the final model for overall survival only (p=0.001). In the final model for overall survival, the effect of *NPM1* appeared to be increasing linearly during the first 6 months, remaining constant thereafter. Allowing a time-dependent effect for *NPM1* (linear during the first 6 months, and constant thereafter) did result in the same variable selection with similar regression coefficients for the other covariables, and a slightly larger effect for *NPM1* mutation. Therefore, the simpler model with constant effect of *NPM1* was chosen as the best approximation to model the effect of *NPM1* for both long-term and short-term survival. In the final Cox model, the highest variance inflation factor was 1.4 (for WBC). Therefore, there was no concern for collinearity between the regressors.

We aimed to derive three groups of low (LowR), intermediate (IntR), and high risk (HiR) to be able to identify patients with good, intermediate, or poor prognosis, but not more, in order not to overfit the model to our data by defining numerically small risk groups. To achieve this goal, pairs of potential cut-off values for the prognostic score defining risk groups were assessed between the 15% and 85% quantiles in steps of 0.1. In order to avoid a similar outcome of two risk groups we required that the ratio between the two Wald statistics of IntR versus LowR and HiR versus IntR groups ranged between 2/3 and 3/2. In the set of pairs of cut-off values for the prognostic score fulfilling these two conditions we selected the one defining risk groups with the maximal log rank statistic.

For internal validation we used the "more refined bootstrap approach"[12] to estimate hazard ratios (HR) with 95% confidence intervals (CI) between risk groups corrected for overfitting. On each of 999 bootstrap samples, drawn with replacement from the full sample, we fitted the multivariable Cox model to determine a prognostic score and selected pairs of cut-off values defining risk groups using the previously described methods. We then applied the prognostic score and the cut-off values determined on the bootstrap sample to the bootstrap sample itself and to the full sample to define LowR, IntR, and HiR groups, and calculated the difference of the log-HR for IntR versus LowR, and HiR versus IntR, respectively, between bootstrap and full sample. The average difference from 999 bootstrap samples ("optimism") was then added to the ("optimistic") log-HR estimated from the full model with the original cut-off values for the original prognostic score and the sum was exponentiated to get the optimism-corrected HR **(Table S4)**. Similarly, we estimated the c-index for prognostic discrimination with and without

bootstrap-correction for overfitting[13].

Relapse-free survival was defined as the time to the competing events relapse or death in CR. To evaluate the ability of the prognostic index for CN-AML (PINA) for RFS ($PINA_{RFS}$) to distinguish risk groups for time to relapse and time to death in CR separately, a competing risk analysis was performed treating relapse, death in CR, and allogeneic transplantation in CR as competing events. Herein, we calculated cumulative incidence rates[14] and HR[15] for relapse according to the $PINA_{RFS}$ risk groups. Cumulative incidence rates between risk groups were compared by Gray's test[16]. Statistical analyses for the AMLCG99 patients were performed using SPSS version 20.0 (SPSS Inc Chicago, ILL USA) and R version 2.12.0 (R Foundation for Statistical Computing; www.r-project.org). Statistical analyses for the CALGB cohort were done using SAS 9.3 (SAS Institute Inc., Cary, NC, USA) and S+ (TIBCO Spotfire S+ Version 8.2.0).

RESULTS

Univariable Cox regression for OS and RFS

Patient characteristics with univariable impact on OS and RFS were age, WBC, LDH level, ECOG performance status 2/3/4 versus 0/1, origin of AML, mutations of *NPM1*, *FLT3*-ITD, and biallelic *CEBPA* mutations (bi*CEBPA*). Hemoglobin level, platelet count, bone marrow blasts, peripheral blasts, sex, monoallelic *CEBPA* mutations (mo*CEBPA),* and type of induction therapy did not have impact on OS or RFS **(Table S2)**.

Multivariable Cox regression for OS and RFS

Since the peripheral blast count was available in <90% of patients it was excluded from further analyses. However, explorative multivariable Cox regression analyses for OS and RFS revealed that peripheral blast count was not of additional prognostic impact.

The ECOG performance status was divided in two groups: asymptomatic or able to carry out light work (ECOG 0-1) and unable to work or confined to bed (ECOG 2-4), since there were no relevant differences between ECOG 0 versus 1, and between ECOG 2 versus 3 versus 4, in Cox regression models. Furthermore, only 42 of 655 patients (6%) had ECOG 3-4.

Therapy with TAD/HAM versus HAM/HAM was exploratively introduced as a parameter in multivariable Cox regression models for OS and RFS, but was not prognostic (p=0.492 for OS, p=0.764 for RFS).

Prognostic value of PINA in patients not used for model development

In the model development we had to exclude patients with a missing value for any of the candidate prognostic factors. Of these patients, 71 and 53 patients had evaluable PINA for OS ($PINA_{OS}$) and $PINA_{RFS}$, respectively. As sensitivity analyses to judge a potential selection bias, we checked the prognostic value of $PINA_{OS}$ and $PINA_{RFS}$ in these patients. Although patient numbers were small, both indices were highly prognostic in the patients not used for model development (overall $p<0.001$ and $p=0.005$, respectively, **Figures S2, S3**).

Adjusted risk groups for OS in patients <60 years

According to the $PINA_{OS}$ only 4 patients <60 years were grouped as HiR **(Figure 3A).** Univariable Cox regression analyses for OS in which the continuous $PINA_{OS}$ score as a variable itself was applied separately in patients <60 years [HR 3.1 (95% CI: 2.3-4.4)] and ≥60 years [HR 2.7 (95% CI: 2.1-3.4)] revealed that the $PINA_{OS}$ score was similarly prognostic in both age groups. To further refine the risk stratification in younger patients, we searched for age-adjusted cut-off values for the $PINA_{OS}$ score using the previously described strategy in the cohort of patients <60 years. This strategy resulted in a low risk group identical to the one defined in the total cohort (cut-off value 4.0) and a 18% poor risk group (cut-off value 4.6). Five-year OS according to the so-defined age-adjusted risk groups were 82% versus 47% versus 18% **(Figure S4)**.

Cumulative incidence of relapse, death and transplantation in patients with a CR

Of 381 patients achieving a CR in which $PINA_{RFS}$ was available, 42 patients underwent allogeneic transplantation in first CR, 188 patients relapsed, and 35 patients died in CR without transplantation **(Figure S5A)**. The application of the $PINA_{RFS}$ led to discrimination of three different risk groups relative to the cumulative incidence of relapse **(Figure S5B)**.

Application of the $PINA_{OS}$ on event-free survival

Event-free survival (EFS) was defined as the period from the start of therapy until lack of a complete remission (CR), relapse after CR or death in CR. According to the $PINA_{OS}$ 5-year EFS in LowR, IntR, and HiR groups was 46%, 15%, and 2% respectively ($p<0.001$) with a HR of 2.4 (95% CI, 1.9-3.2) for IntR versus LowR and 2.0 (95% CI, 1.5-2.5) for HiR versus IntR **(Figure S6)**.

Distribution of clinical and molecular markers in risk groups

Most clinical and molecular markers were differently distributed in the $PINA_{OS}$ and the $PINA_{RFS}$ risk groups **(Table S5)** reflecting their prognostic impact. A subset of 42% of the molecularly favorable *NPM1*+/*FLT*3-ITD- group was classified as IntR group according to the $PINA_{OS}$. Of the LowR group 41% were not *NPM1* positive/*FLT*3-ITD negative, and 23% of patients in the IntR group were *NPM1*+/*FLT*3-ITD-.

Patient characteristics and outcome used for external validation (CALGB trials)

In the validation cohort of 529 patients from CALGB trials all patients had de novo AML. Median age was 58 years (19-89 years) and 47% were older than 60 years; 82% had an ECOG performance status ≤1. Median bone marrow blasts were 65%. Mutations of *NPM1*, *FLT3*-ITD, and *CEBPA* were present in 61%, 35%, and 16% of patients, respectively.

Per protocol, patients did not receive allogeneic transplantation in first CR.

The median follow-up for OS of patients alive was 7.9 years; 400 of the 529 patients died. The median OS was 1.4 years and the median EFS was 0.8 years. 402 patients (76%) achieving a CR had a median RFS of 1.2 years; 298 of the 403 patients relapsed or died **(Table S7).**

External validation of the $PINA_{OS}$ and the $PINA_{RFS}$ in patients <60 years and ≥60 years

In 279 patients <60 years of the CALGB cohort, the $PINA_{OS}$ defined a LowR group (63% of patients) with a 5-year OS of 51% and an IntR group with a 5-year OS of 35% (HR: 2.1; 95% CI: 1.6-2.9) **(Figure S7A).** Only 4 patients were assigned to the HiR group.

In 250 patients aged ≥60 years, 9%, 67%, and 24% of the patients were classified as LowR, IntR, and HiR according to the $PINA_{OS,}$ and 5-year OS rates were 53%, 13%, and 3% **(Figure S7B).** The HRs for IntR versus LowR and HiR versus IntR were 2.5 (95% CI: 1.4-4.2) and 1.6 (95% CI: 1.2-2.2), respectively.

In 234 patients <60 years who achieved a CR the $PINA_{RFS}$ distinguished a LowR group (53% of patients), where the 5-year RFS was 56%, from an IntR group (39% of patients) with a 5-year RFS of 24% (HR: 2.7; 95% CI: 1.9.-3.8) and a HiR group (8% of patients) (5-year RFS: 6%) (HR compared to the IntR group: 1.5; 95% CI: 0.9-2.6) **(Figure S7C).**

Of the 168 patients ≥60 years who achieved a CR, the $PINA_{RFS}$ classified 42%, 23%, and 35% as LowR, IntR, and HiR, with 5-year RFS rates of 18%, 20%, and 3% respectively **(Figure S7D).** The HRs for RFS comparing IntR versus LowR and HiR versus IntR were 1.3 (95% CI: 0.9-2.0) and 1.7 (95% CI: 1.2-2.5) respectively.

SUPPLEMENTARY FIGURES

Figure S1: Overview of patient selection

AMLCG, AML Cooperative Group; CALGB, Cancer and Leukemia Group B; CN-AML, cytogenetically normal acute myeloid leukemia; CR, complete remission; MDS, myelodysplastic syndrome; OS, overall survival; $PINA_{OS}$, prognostic index for CN-AML for OS; $PINA_{RFS}$, prognostic index for CN-AML for RFS; RFS, relapse-free survival.

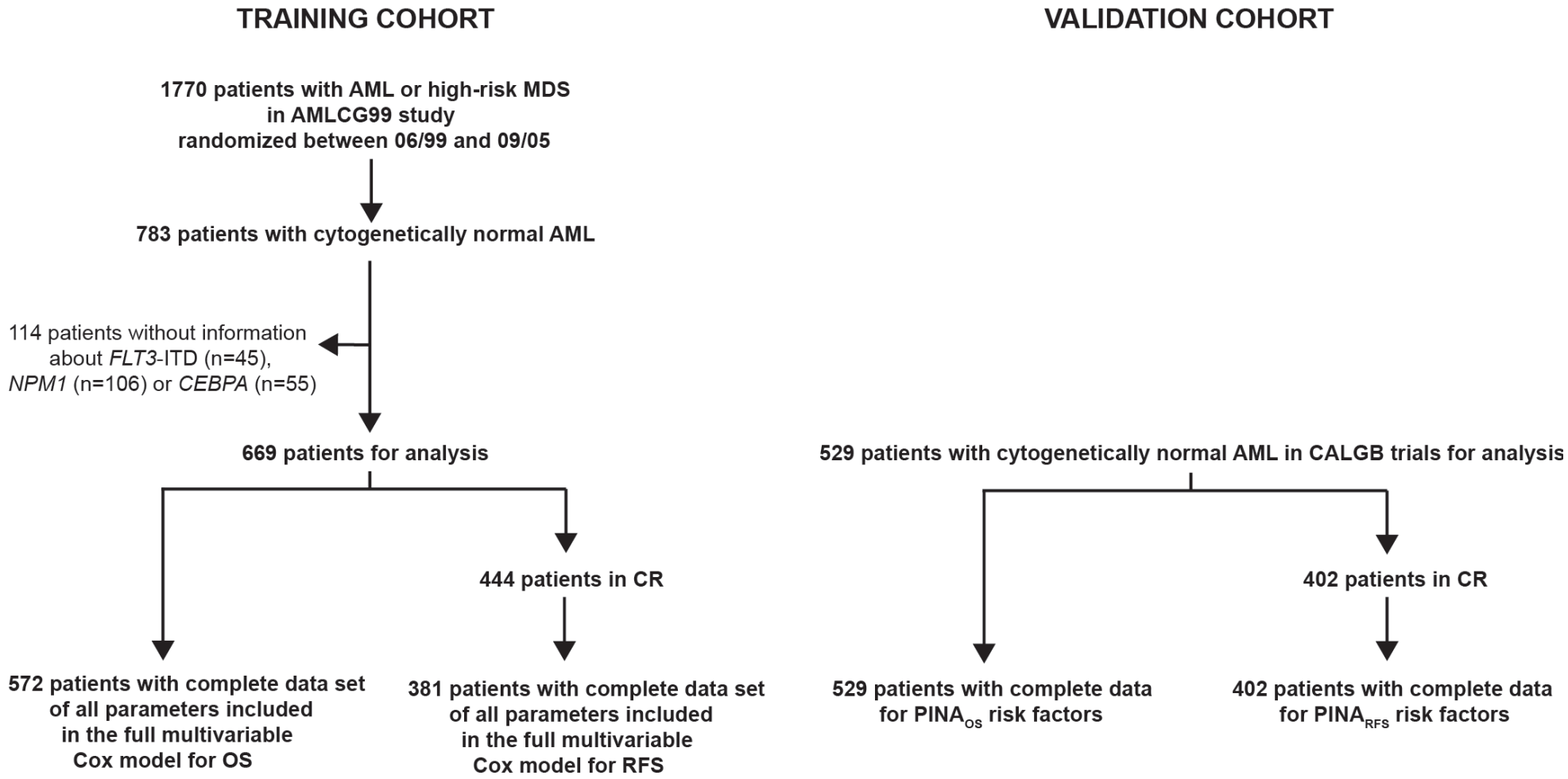


Figure S2: Overall survival (OS) according to the prognostic index for cytogenetically normal acute myeloid leukemia for OS ($PINA_{OS}$) in 71 patients not used for model development due to missing values of variables excluded by backward elimination. CI, confidence interval; HiR, high risk; HR, hazard ratio; IntR, intermediate risk; LowR, low risk.

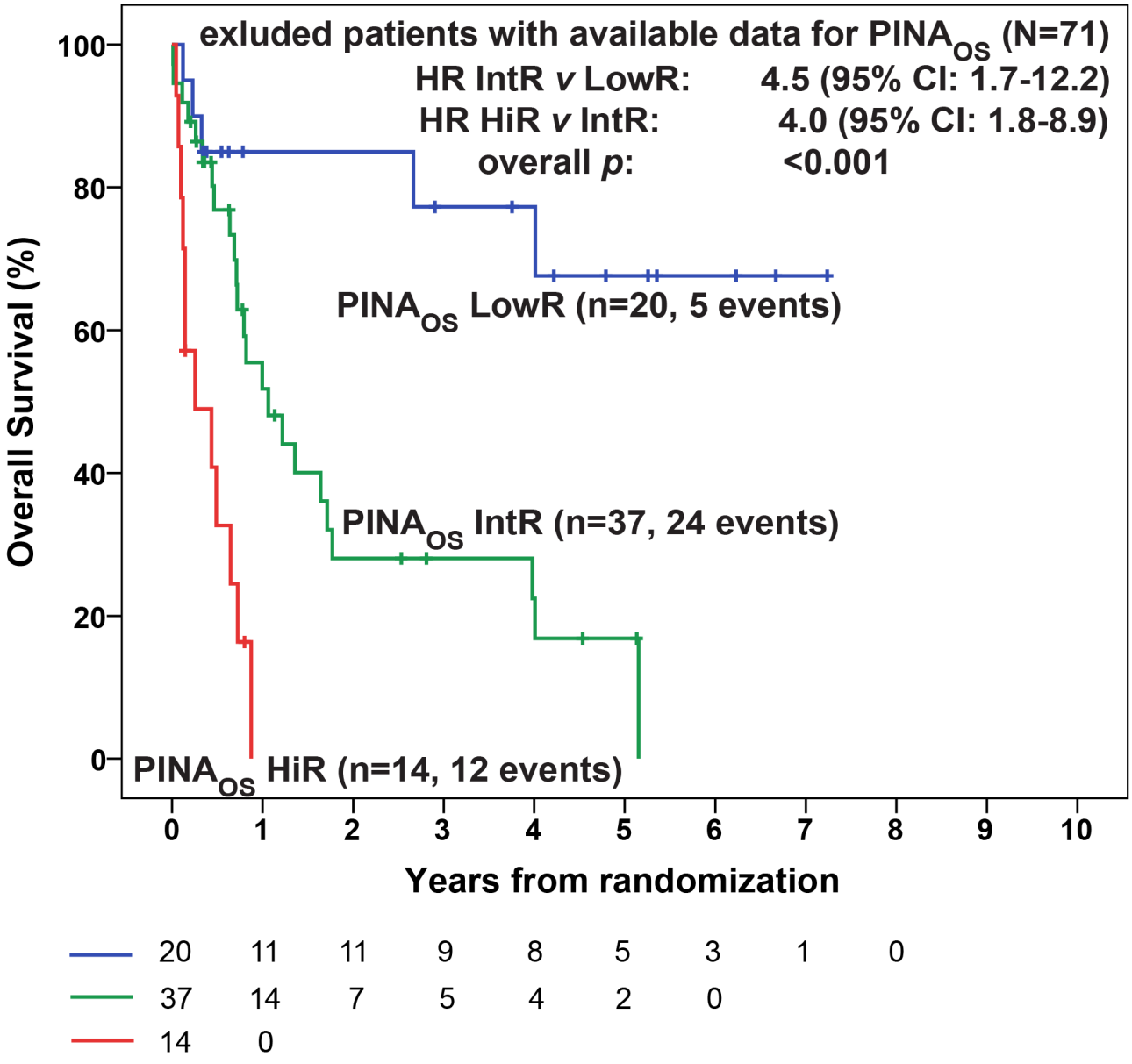


Figure S3: Relapse-free survival (RFS) according to the prognostic index for cytogenetically normal acute myeloid leukemia for RFS ($PINA_{RFS}$) in 53 patients with complete remission not used for model development due to missing values of variables excluded by backward elimination. CI, confidence Interval; CR, complete remission; HiR, high risk; HR, hazard ratio; IntR, intermediate risk; LowR, low risk.

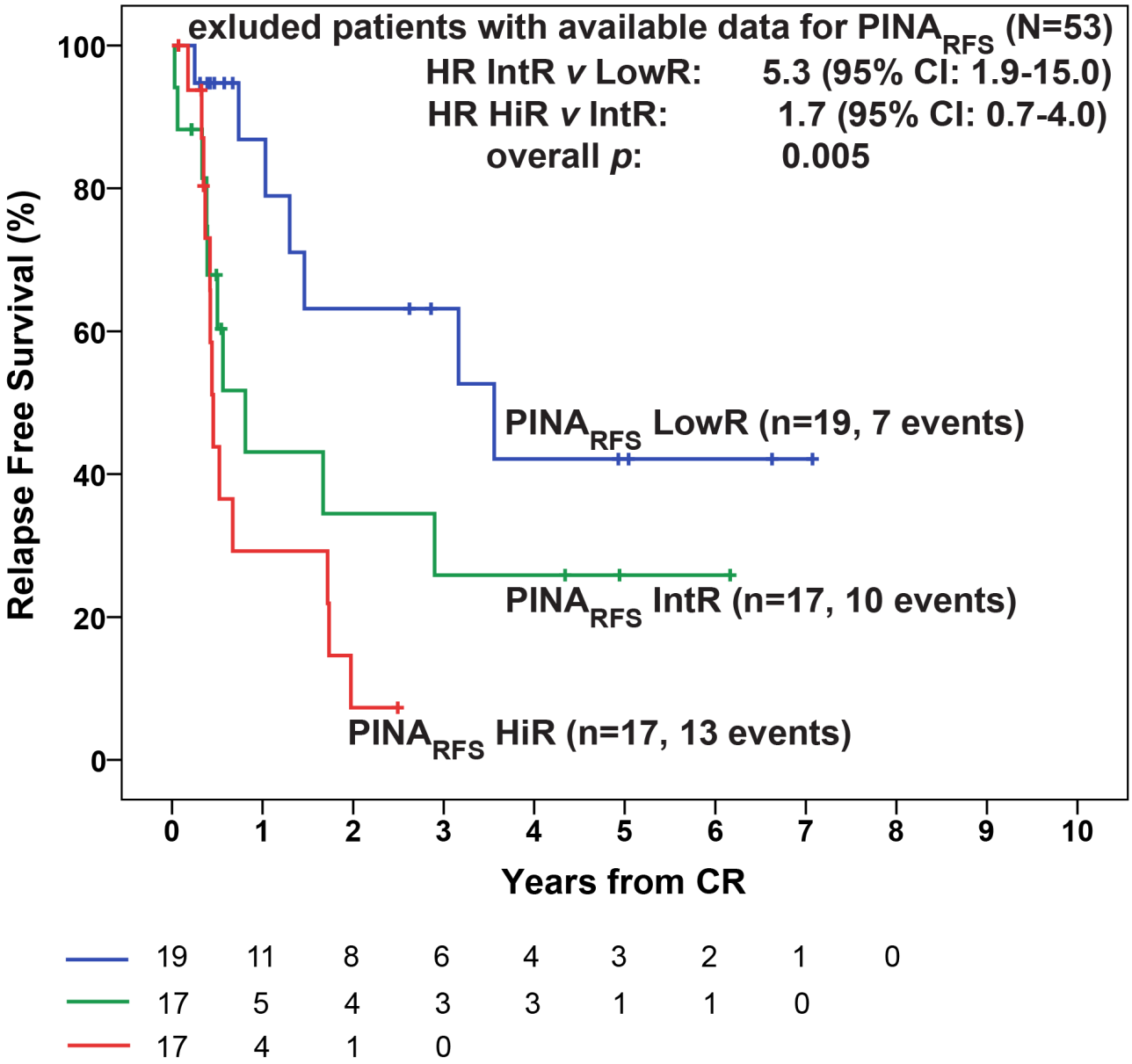


Figure S4: Overall survival (OS) according to age-adjusted cutoff values (4.0 and 4.6) for the prognostic index for cytogenetically normal acute myeloid leukemia for OS ($PINA_{OS}$) score in patients <60 years. CI, confidence interval, HR, hazard ratio.

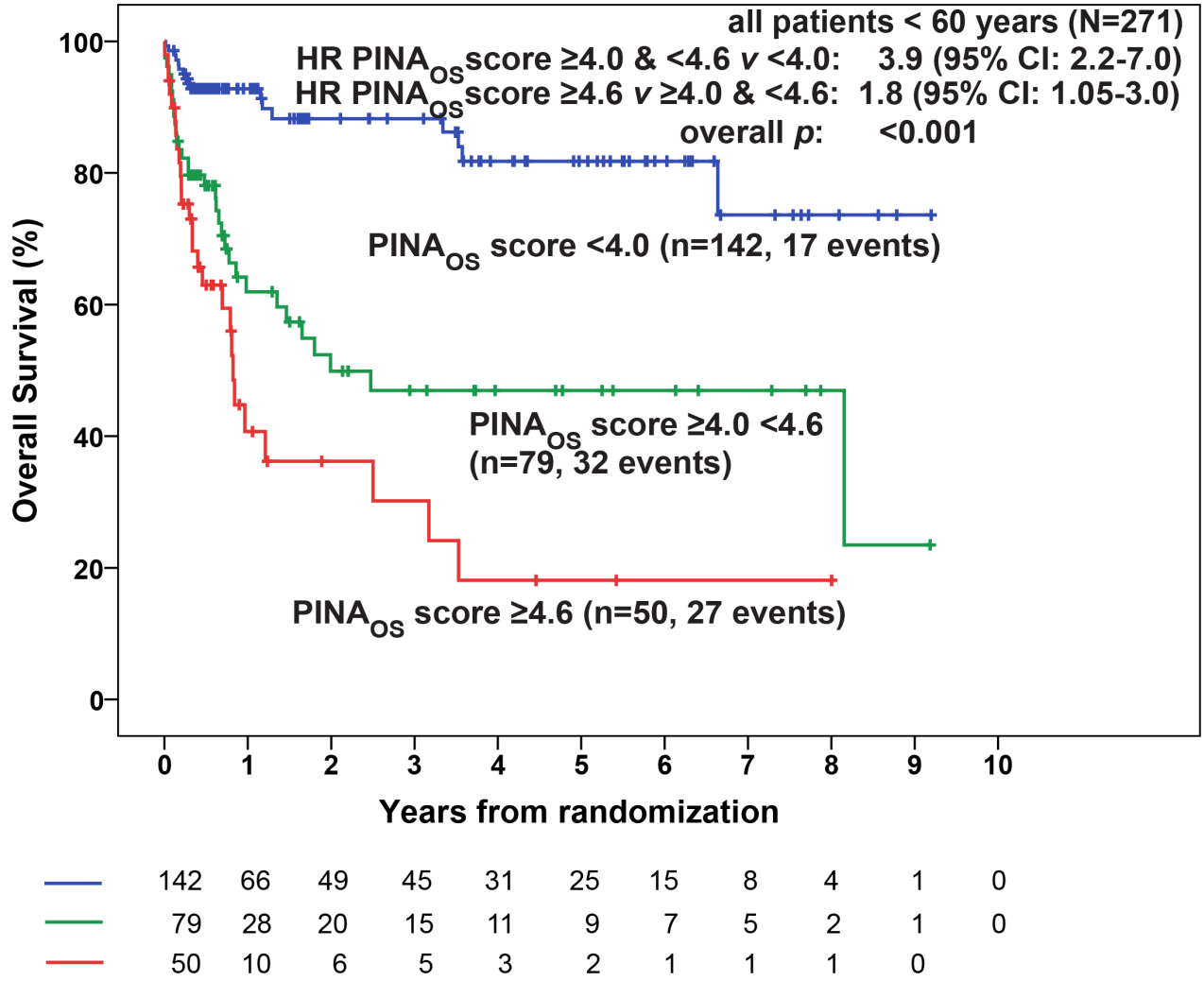
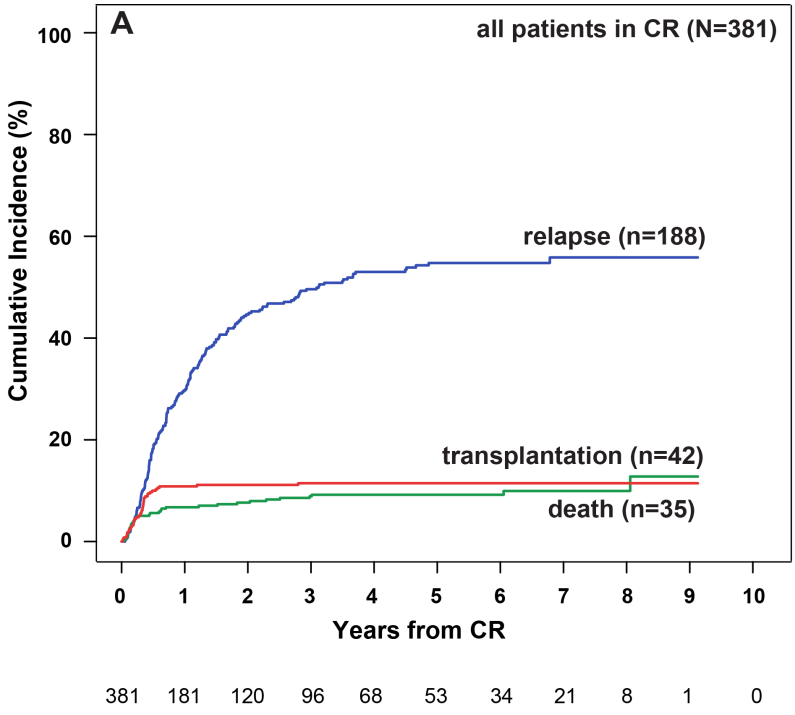


Figure S5: Cumulative incidence of relapse, death without relapse and allogeneic transplantation in 381 patients with a CR in the entire cohort (A) and cumulative incidence of relapse in subgroups separated by the prognostic index for cytogenetically normal acute myeloid leukemia for RFS ($PINA_{RFS}$) (B). CI, confidence interval; CR, complete remission; HiR, high risk; HR, hazard ratio; IntR, intermediate risk; LowR, low risk.



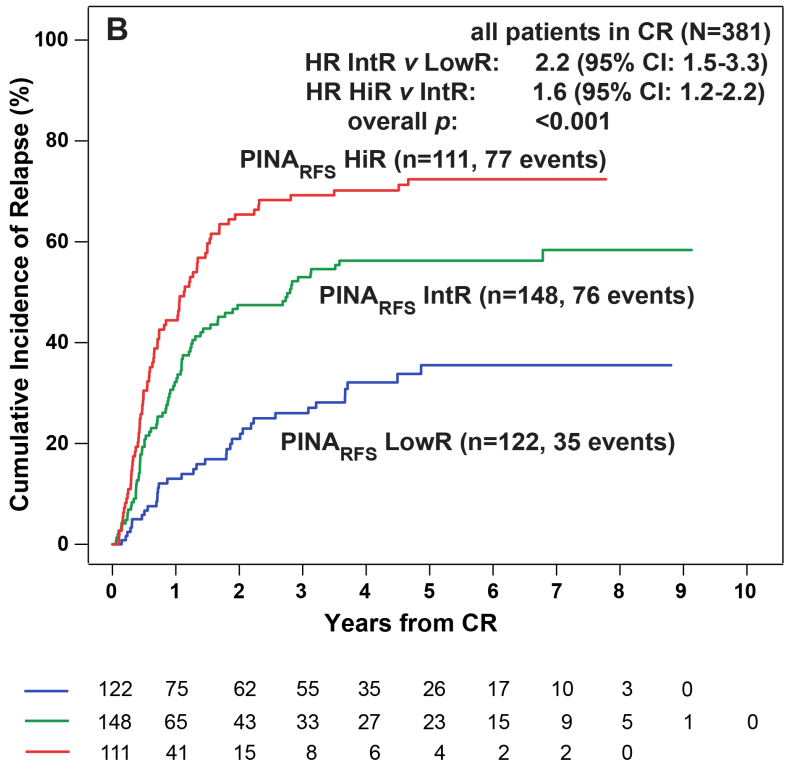


Figure S6: Event-free survival (EFS) according to the prognostic index for cytogenetically normal acute myeloid leukemia for OS ($PINA_{OS}$) risk groups. CI, confidence interval; HiR, high risk; HR, hazard ratio; IntR, intermediate risk; LowR, low risk.

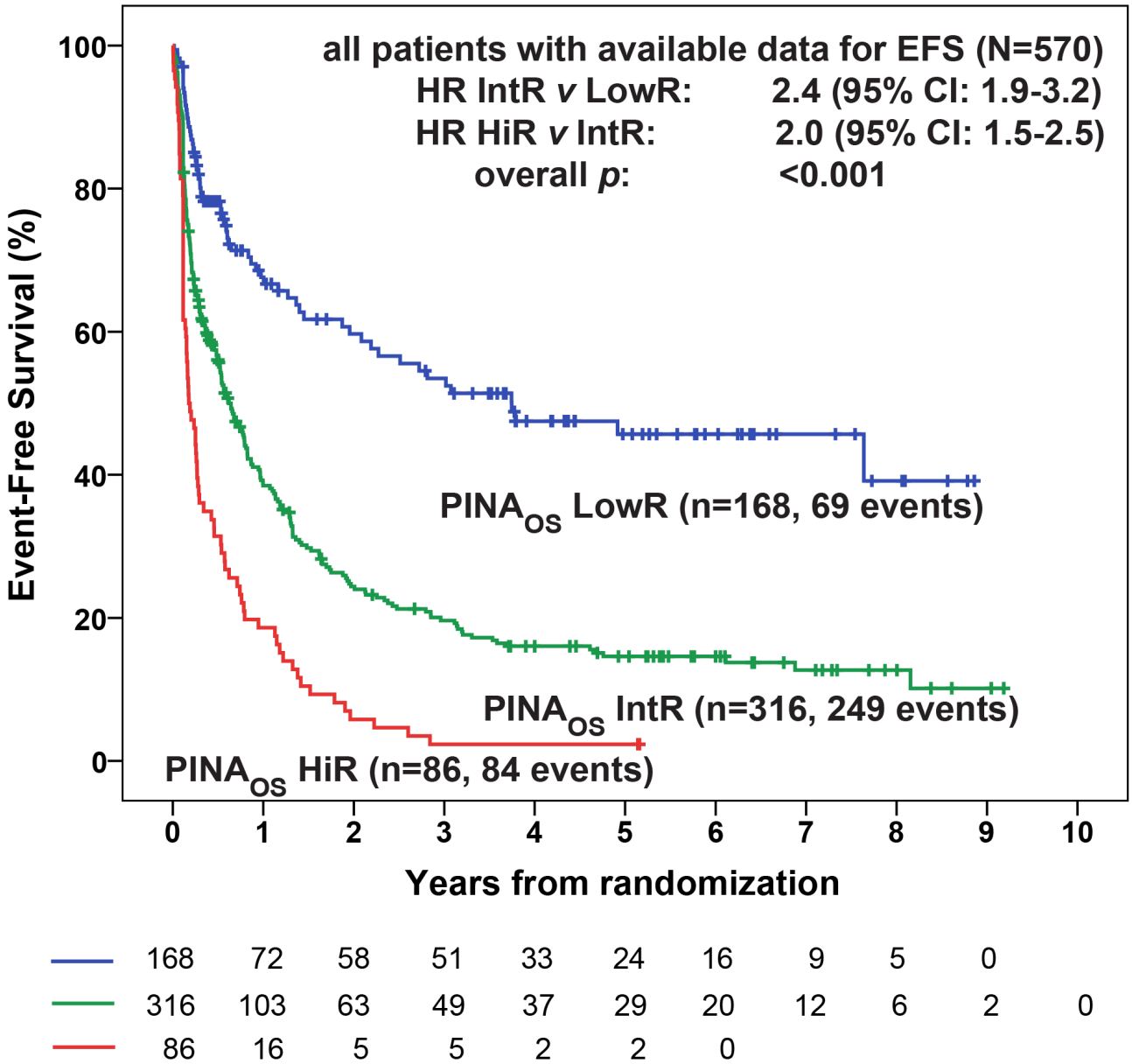
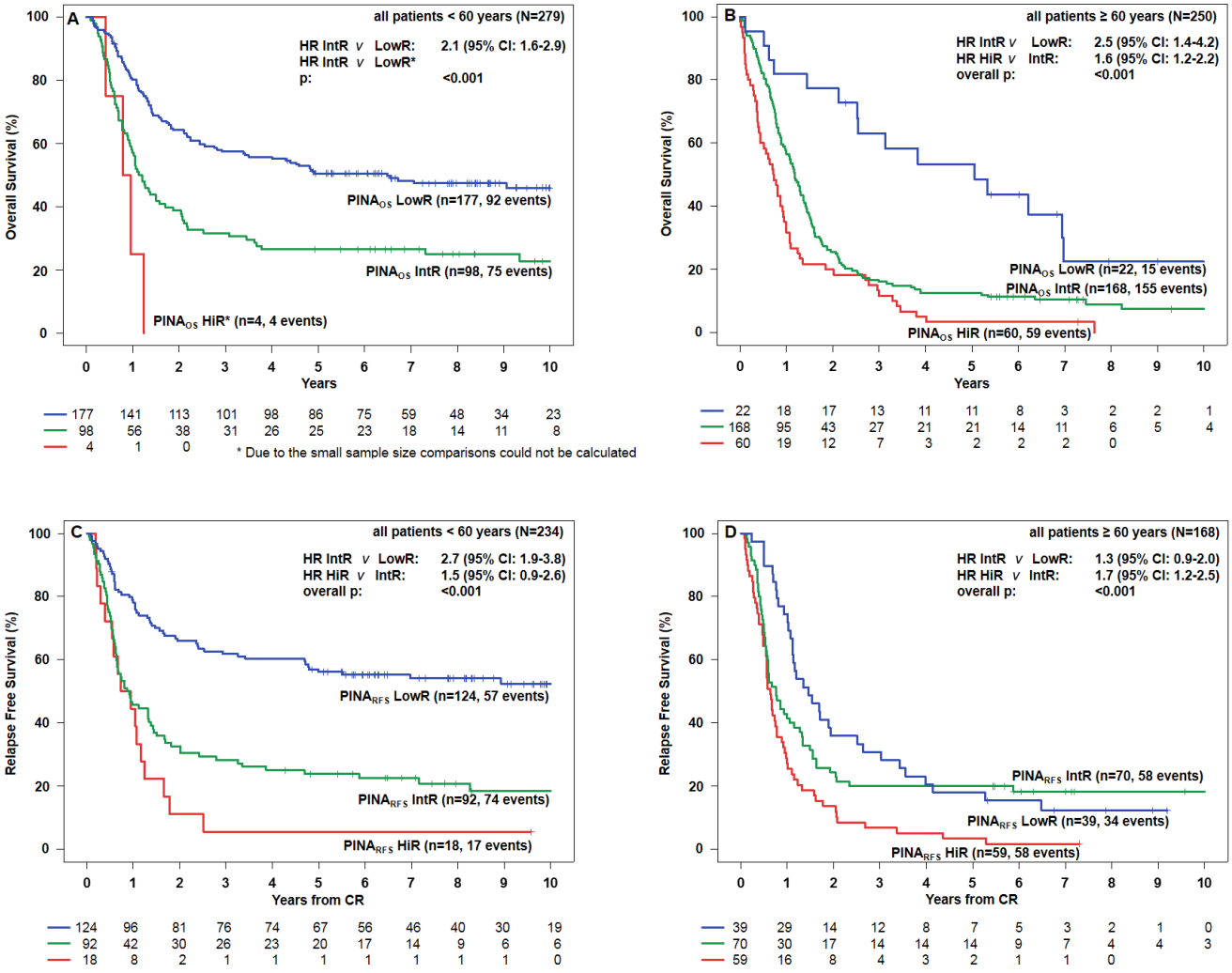


Figure S7: Outcome according to the new prognostic indices in the CALGB validation cohort in cytogenetically normal acute myeloid leukemia patients. (A) Overall survival (OS) according to the prognostic index for CN-AML for OS ($PINA_{OS}$) in patients <60 years, (B) relapse-free survival (RFS) according to the prognostic index for CN-AML for RFS ($PINA_{RFS}$) in patients <60 years, (C) OS according to $PINA_{OS}$ in patients ≥60 years, (D) RFS according to $PINA_{RFS}$ in patients ≥60 years. CI, confidence interval; CR, complete remission; HiR, high risk; HR, hazard ratio; IntR, intermediate risk; LowR, low risk.



SUPPLEMENTARY TABLES

Table S1: Characteristics and clinical outcome in 669 patients with CN-AML in AMLCG99 trial

Characteristic	n	%
Age, years		
median	60	
range	17-85	
WBC, G/L (n=662)		
median	18.7	
range	0.1-798	
Platelets, G/L(n=662)		
median	58	
range	5-643	
Hemoglobin level, g/L (n=659)		
median	92	
range	42-164	
LDH level, U/L (n=654)		
median	422	
range	102-14332	
Bone marrow blasts, % (n=663)		
median	80	
range	20-100	
Peripheral blasts, % (n=547)		
median	40	
range	0-99	
Female sex	336	50
Performance status (ECOG) (n=655)		
0	165	25
1	297	45
2	151	23
3	33	5
4	9	1
Origin of AML		
de novo	563	84
sAML	87	13
tAML	19	3
NPM1+	345	52
FLT3-ITD+	194	29
NPM1+/FLT3-ITD-	205	31
mo*CEBPA*+	28	4
bi*CEBPA*+	31	5
ELN genetic group		
Favorable	256	38
Intermediate-I	413	62
Induction regimen (n=666)		
TAD	108	16
HAM	134	20
TAD-HAM	230	34
HAM-HAM	194	29
Allogeneic transplantation	124	19
Time to transplantation, months (n=124)		
median	6.5	
range	2.7-37	
OS, years		
median	1.9	
events	321	48
median follow-up	3.7	
CR	444	66

Characteristic	n	%
RFS, years (n=442)		
median	1.5	
events	242	55
type of event: relapse after CR	205	
type of event: death in CR	37	

Between the selected (669) and the nonselected (114) patients there was no difference in OS (median 1.9 versus 1.6 years, p=0.31), EFS (0.7 versus 0.7 years, p=0.36) and RFS (1.5 versus 1.4 years p=0.58).

Abbreviations: AML, acute myeloid leukemia; bi*CEBPA*+, biallelic mutation of the CCAAT/enhancer-binding protein alpha gene; CN, cytogenetically normal; CR, complete remission; ELN, European Leukemia Net; ECOG, Eastern Cooperative Group; *FLT*3-ITD+, presence of an internal tandem duplication of the *FLT*3 gene; *FLT*3-ITD-, absence of an internal tandem duplication of the *FLT*3 gene; HAM, high dose cytarabine, mitoxantrone; LDH, lactate dehydrogenase; n, number; mo*CEBPA*+, monoallelic mutation of the CCAAT/enhancer-binding protein alpha gene; *NPM1*+, mutation in the nucleophosmin 1 gene; OS, Overall survival; RFS, Relapse-free survival; sAML, secondary AML; TAD, thioguanine, cytarabine, daunorubicin; tAML, therapy-related AML; WBC, white blood count.

Table S2: Univariable Cox regression for overall survival (OS) and relapse-free survival (RFS)

		OS				RFS			
Variable	Comparison	n	Hazard Ratio	95% CI	p	n	Hazard Ratio	95% CI	p
Age (years)	+10 years	669	1.6	1.4 -1.7	<0.001	442	1.2	1.1-1.4	<0.001
WBC (10^6/L)	10 fold	662	1.3	1.1-1.6	<0.001	437	1.2	0.980-1.419	0.08
Platelets (10^6/L)	10 fold	662	0.8	0.6-1.0	0.07	437	0.8	0.5-1.044	0.09
Hb (g/L)	+1 g/L	659	1.0	0.991-1.003	0.32	436	1.0	0.991-1.004	0.48
LDH level (U/L)	10 fold	654	1.7	1.3-2.4	<0.001	428	1.7	1.1-2.5	0.014
BM blasts (%)	+1%	633	1.0	0.996-1.006	0.62	413	1.0	0.994-1.006	0.98
Peripheral blasts (%)*	+1%	505	1.2	0.9-1.5	0.16	326	1.1	0.8-1.4	0.72
Sex	female *v* male	669	1.0	0.9-1.1	0.56	442	1.1	0.9-1.2	0.27
Performance status (ECOG)	1 *v* 0	655	1.3	0.999-1.8	0.051	433	1.2	0.9-1.7	0.21
	2-4 *v* 0		2.0	1.5-2.7	<0.001		1.6	1.1-2.2	0.010
Performance status (ECOG)	2-4 *v* 0,1	655	1.7	1.4-2.1	<0.001	433	1.4	1.053-1.8	0.020
Origin of AML	de novo *v* non de novo	669	0.7	0.5-0.9	0.005	442	0.8	0.6-1.1	0.11
NPM1	mutated *v* wt	669	0.6	0.5-0.7	<0.001	442	0.5	0.4-0.7	<0.001
FLT3-ITD	pos. *v* neg.	669	1.4	1.1-1.7	0.006	442	1.7	1.3-2.3	<0.001
CEBPA	mo*CEBPA*+ *v* wt	669	1.0	0.6-1.7	0.94	439	0.9	0.4-1.8	0.75
CEBPA	bi*CEBPA*+ *v* wt	669	0.4	0.2-0.8	0.008	439	0.5	0.3-0.967	0.039
ELN genetic group	Intermediate-I *v* favorable	669	2.3	1.8-2.9	<0.001	442	3.1	2.3-4.1	<0.001
Induction therapy	TAD-HAM *v* HAM-HAM	669	1.1	0.9-1.4	0.30	442	1.0	0.8-1.3	0.74

* not considered for multivariable regression

Abbreviations: bi*CEBPA*+, biallelic mutation of the CCAAT/enhancer-binding protein alpha gene; BM blasts, bone marrow blasts; *CEBPA*, CCAAT/enhancer-binding protein alpha; CI: confidence interval; ECOG, performance status according to the Eastern Cooperative Oncology Group; ELN, European Leukemia Net; *FLT3*-ITD, internal tandem duplication of the *FLT3* gene; HAM, high dose cytarabine, mitoxantrone; Hb, hemoglobin; LDH, lactate dehydrogenase; mo*CEBPA*+, monoallelic mutation of the CCAAT/enhancer-binding protein alpha gene; neg., negative; *NPM1*, nucleophosmin 1; OS, Overal survival; pos., positive; RFS, Relapse-free survival; WBC, white blood count; wt, wild-type.

Table S3: Characteristics of patients with a complete data set used for the establishment of the PINA

Characteristic	$PINA_{OS}$ Score (n=572)	$PINA_{RFS}$ Score (n=381)
Age, years		
median	61	59
range	17-83	17-78
White blood count, G/L		
median	18.5	16.0
range	0.1-798	0.5-785.5
Platelets, G/L		
median	60	60
range	5-643	5-623
Hemoglobin level, g/L		
median	92	92
range	42-164	42-148
LDH level, U/L		
median	421	408
range	102-14332	121-7434
Bone marrow blasts, %		
median	80	80
range	20-100	20-100
Peripheral blasts, %*		
median	41	39
range	0-99	0-98
Female sex, n (%)	287 (50)	198 (52)
Performance status (ECOG), n (%)		
0	140 (25)	98 (26)
1	258 (45)	180 (47)
2	138 (24)	81 (21)
3	30 (5)	19 (5)
4	6 (1)	3 (1)
Origin of AML, n (%)		
de novo	483 (84)	333 (87)
sAML	73 (13)	38 (10)
tAML	16 (3)	10 (3)
NPM1+, n (%)	297 (52)	222 (58)
FLT3-ITD+, n (%)	174 (30)	117 (31)
NPM1+/FLT3-ITD-, n (%)	175 (31)	135 (35)
mo*CEBPA*+, n (%)	26 (5)	15 (4)
bi*CEBPA+,* n (%)	26 (5)	20 (5)
ELN genetic group, n (%)		
Favorable	223 (39)	167 (44)
Intermediate-I	349 (61)	214 (56)
Induction regimen, n (%)		
TAD	94 (16)	57 (15)
HAM	119 (21)	68 (18)
TAD-HAM	196 (34)	145 (38)
HAM-HAM	161 (28)	111 (29)
OS, years		
median	2.1	
events, n (%)	278	
Median follow up	3.7	
EFS, years		
median	0.7	
events, n (%)	402 (71)	
CR, n (%)	381 (67)	
RFS, years		
median		1.5
events, n (%)		213 (56)

Between the 572 patients included in multivariable Cox regression for OS and the 97 excluded patients there was a tendency towards shorter OS for the excluded patients (median 2.1 versus 1.0 years, p=0.07), but comparable EFS (median 0.7 versus 0.6 years, p=0.24) and RFS (median 1.5 versus 1.5 years, p=0.55).

Abbreviations: bi*CEBPA*+, biallelic mutation of the CCAAT/enhancer-binding protein alpha gene; CR, complete remission; ECOG, performance status according to the Eastern Cooperative Oncology Group; EFS, Event-free survival; *FLT*3-ITD+, presence of an internal tandem duplication of the *FLT*3 gene; *FLT*3-ITD-, absence of an internal tandem duplication of the *FLT*3 gene; HAM, high-dose cytarabine, mitoxantrone; LDH, lactate dehydrogenase; mo*CEBPA*+, mutation of the CCAAT/enhancer-binding protein alpha gene; n, number; *NPM1*+, mutation in the nucleophosmin 1 gene; OS, Overall survival; PINA, prognostic index in cytogenetically normal acute myeloid leukemia; RFS, Relapse-free survival; sAML, secondary AML; TAD, thioguanine, cytarabine, daunorubicin; tAML, therapy-related AML.

*Number of patients with available information of peripheral blasts for $PINA_{OS}$ 475/572 and $PINA_{RFS}$ 314/381

Table S4: Hazard ratios and c-index[13] for OS between $PINA_{OS}$ risk groups and for RFS between $PINA_{RFS}$ risk groups corrected for overfitting (bootstrap validation)

Estimation method	Comparison	$PINA_{OS}$ Hazard Ratio	95% CI	c -index	$PINA_{RFS}$ Hazard Ratio	95% CI	c -index
Optimistic*	IntR *v* LowR	4.4	3.0-6.6	0.6712	2.6	1.8-3.9	0.6645
	HiR *v* IntR	2.5	1.9-3.2		2.0	1.5-2.7	
Bootstrap-corrected	IntR *v* LowR	4.1	2.7-5.6	0.6611	2.4	1.5-3.4	0.6500
	HiR *v* IntR	2.3	1.8-2.9		1.8	1.3-2.4	

*Optimistic estimation method: calculation on the data set used for model development.

Abbreviations: CI, confidence interval; HiR, high risk; IntR, intermediate risk; LowR, low risk; OS, Overall survival; PINA, prognostic index in cytogenetically normal acute myeloid leukemia; RFS, Relapse-free survival.

Table S5: Patient characteristics (AMLCG cohort) in risk groups according to the $PINA_{OS}$ and $PINA_{RFS}$

Characteristic	$PINA_{OS}$ (n=572) LowR n=168	IntR n=318	HiR n=86	*P*	$PINA_{RFS}$ (n=381) LowR n=122	IntR n=148	HiR n=111	*P*
Age (years)								
median	45	62	69	<0.001	52	56	66	<0.001
range	17-76	29-83	51-80		19-77	17-78	29-78	
WBC (G/L)								
median	9.3	18.0	49.3	<0.001	10.2	30.9	18.6	0.016
range	0.1-798.2	0.5-785.5	2.2-440.3		0.5-141.0	0.5-785.5	1.1-486.0	
Platelets (G/L)								
median	63	60	47	0.025	70	58	54	0.20
range	5-339	5-643	8-458		5-339	6-623	7-471	
Hemoglobin level (g/L)								
median	92	93	93	0.63	91	93	91	0.88
range	47-146	45-164	42-156		47-147	45-136	42-148	
LDH (U/L)								
median	365	424	576	0.001	362	423	443	0.10
range	132-2821	102-14332	161-5520		132-2067	122-14332	121-7434	
BM blasts (%)								
median	80	80	80	0.58	80	80	80	0.30
range	20-100	20-100	20-100		20-100	20-100	20-100	
Peripheral blasts (%)[#]								
median	31	39	67	<0.001	35	40	50	0.31
range	0-98	0-99	0-99		0-98	0-97	0-98	
Female sex (n, %)	93 (55)	155 (49)	39 (45)	0.24	67 (55)	81 (55)	50 (45)	0.22
ECOG 2-4 (n, %)	24 (14)	92 (29)	58 (67)	<0.001	30 (25)	38 (26)	36 (32)	0.44
de novo (n, %)	155 (92)	260 (82)	68 (79)	0.003	115 (94)	125 (85)	93 (84)	0.021
NPM1+ (n, %)	125 (74)	150 (47)	23 (27)	<0.001	110 (90)	84 (57)	26 (23)	<0.001
*FLT*3-ITD+ (n, %)	33 (20)	105 (33)	34 (38)	0.002	10 (8)	62 (42)	41 (37)	<0.001
NPM1+/*FLT*3-ITD- (n, %)	99 (59)	74 (23)	2 (2)	<0.001	103 (85)	31 (22)	0 (0)	<0.001
mo*CEBPA*+ (n, %)	5 (3)	14 (4)	7 (8)	0.17	3 (3)	5 (3)	7 (6)	0.29
bi*CEBPA*+ (n, %)	16 (10)	10 (3)	0 (0)	0.001	11 (9)	9 (6)	0 (0)	0.007
ELN genetic group								
Favorable	118 (70)	97 (30)	8 (9)	<0.001	114 (93)	46 (31)	7 (6)	<0.001
Intermediate-I	50 (30)	221 (70)	78 (91)		8 (7)	102 (28)	104 (94)	
Induction regimen[+] (n; %)								
TAD	10 (6)	66 (21)	18 (21)	<0.001	21 (17)	20 (14)	16 (14)	0.009
HAM	15 (9)	66 (21)	38 (44)		14 (12)	22 (15)	32 (29)	
TAD-HAM	78 (46)	103 (33)	15 (17)		44 (36)	66 (45)	35 (32)	
HAM-HAM	65 (39)	81 (25)	16 (18)		43 (35)	40 (27)	28 (25)	
Allogeneic transplantation	63 (38)	43 (14)	4 (5)	<0.001	28 (23)	36 (24)	17 (15)	0.19

Abbreviations: bi*CEBPA*+, biallelic mutation of the CCAAT/enhancer-binding protein alpha gene; BM blasts, bone marrow blasts at initial diagnosis; de novo, AML of de novo origin; ECOG, performance status according to the Eastern Cooperative Oncology Group; *FLT*3-ITD+, presence of an internal tandem duplication of the *FLT*3 gene; *FLT*3-ITD-, absence of an internal tandem duplication of the *FLT*3 gene; HAM, high dose cytarabine, mitoxantrone; Hb, hemoglobin level; HiR, high risk; IntR, intermediate risk; LDH, lactase dehydrogenase; LowR, low risk; mo*CEBPA*+, monoallelic mutation of the CCAAT/enhancer-binding protein alpha gene; n, number; *NPM1*+, mutation in the nucleophosmin 1 gene; OS, Overall survival; PINA, prognostic index in cytogenetically normal acute myeloid leukemia; RFS, Relapse-free survival; TAD, thioguanine, cytarabine, daunorubicin; WBC, white blood count.

[#] number with available information about peripheral blasts for $PINA_{OS}$ =475/572 cases; $PINA_{RFS}$: 314/381 cases

[+]number with available information about induction regimen for $PINA_{OS}$ =571/572 cases; $PINA_{RFS}$: 381/381 cases

Table S6: Characteristics of ELN favorable patients (*NPM1*+/*FLT3*-ITD- or *CEBPA*-mutated) according to $PINA_{OS}$ risk groups

Characteristic	$PINA_{OS}$ Group		
	LowR (n=118)	IntR (n=97)	HiR (n=8)
Age (years)			
median	50	67	74
range	18-76	46-83	55-80
WBC (G/L)			
median	13.7	40.2	65.5
range	0.5-798	1.3-786	18.8-192
Platelets (G/L)			
median	65	53	32
range	5-339	9-367	10-137
Hb (g/L)			
median	91	94	85
range	53-146	56-164	68-116
LDH (U/L)			
median	362	474	467
range	132-2821	102-4899	298-2666
BM blasts (%) (n=126)			
median	80	85	83
range	20-100	20-100	70-95
Peripheral blasts (%) (n=205)			
median	36	51	77
range	0-98	0-98	34-93
Female (%)	55	57	75
ECOG 2-4 (%)	14	44	63
de novo (%)	94	93	63
NPM1+ (%)	86	81	38
FLT3-ITD+ (%)	3	7	25
NPM1+/FLT3-ITD- (%)	84	76	25
mo*CEBPA*+ (%)	4	13	75
bi*CEBPA*+ (%)	14	10	0
Induction regimen (%)			
TAD	9	30	38
HAM	13	31	38
TAD-HAM	39	17	25
HAM-HAM	40	23	0

Abbreviations: bi*CEBPA*+, biallelic mutation of the CCAAT/enhancer-binding protein alpha gene; BM blasts, bone marrow blasts at initial diagnosis; de novo, AML of de novo origin; ECOG, performance status according to the Eastern Cooperative Oncology Group; *FLT*3-ITD+, presence of an internal tandem duplication of the *FLT*3 gene; *FLT*3-ITD-, absence of an internal tandem duplication of the *FLT*3 gene; HAM, high dose cytarabine, mitoxantrone; Hb, hemoglobin level; HiR, high risk; IntR, intermediate risk; LDH, lactase dehydrogenase; LowR, low risk; mo*CEBPA*+, monoallelic mutation of the CCAAT/enhancer-binding protein alpha gene; *NPM1*+, mutation in the nucleophosmin 1 gene; OS, Overall survival; PINA, prognostic index in cytogenetically normal acute myeloid leukemia; TAD, thioguanine, cytarabine, daunorubicin; WBC, white blood count.

Table S7: Characteristics and clinical outcome in the CALGB CN-AML validation cohort (529 patients)

Characteristic	n		%
Age, years			
median		58	
range		19-89	
WBC, G/L			
median		24.2	
range		0.6-450	
Platelets, G/L (n=528)			
median		62	
range		4-850	
Hemoglobin level, g/L (n=519)			
median		94	
range		42-251	
Bone marrow blasts, % (n=516)			
median		65	
range		2-99	
Peripheral blasts, % (n=513)			
median		55	
range		0-99	
Female sex	275		52
Performance status (ECOG)			
0	160		30
1	274		52
2	81		15
3	12		2
4	2		1
Origin of AML			
de novo	529		100
sAML	0		0
tAML	0		0
NPM1+	325		61
FLT3-ITD+	185		35
NPM1+/FLT3-ITD-	183		35
mo*CEBPA*+	39		7
bi*CEBPA*+	45		9
ELN genetic group			
Favorable	256		48
Intermediate-I	273		52
OS, years			
median		1.4	
events	400		76
median follow-up for survivors		7.9	
EFS, years			
median		0.8	
events	424		80
CR	402		76
RFS, years (n=403)			
median		1.2	
events	298		74
type of event: relapse in CR	270		67
type of event: death in CR	28		7

Data on lactate dehydrogenase level were not available.

Abbreviations: AML, acute myeloid leukemia; bi*CEBPA*+, biallelic mutation of the CCAAT/enhancer-binding protein alpha gene; CN, Cytogenetically normal; CR, complete remission; ECOG, performance status according to the Eastern Cooperative Oncology Group; EFS, Event-free survival; ELN, European Leukemia Net; *FLT*3-ITD+, presence of an internal tandem duplication of the *FLT*3 gene; *FLT*3-ITD-, absence of an internal tandem duplication of the *FLT*3 gene; LDH, lactate dehydrogenase; mo*CEBPA*+, monoallelic mutation of the CCAAT/enhancer-binding protein alpha gene; *NPM1*+, mutation in the nucleophosmin 1 gene; OS, Overall survival; RFS, Relapse-free survival; sAML, secondary AML; tAML, therapy-related AML; WBC, white blood count.

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